Neuropharmacology 121 (2017) 30–38

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Mitochondrial energy metabolism of rat hippocampus after treatment with the antidepressants desipramine and fluoxetine

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ARTICLE INFO

Article history:
Received 19 November 2016
Received in revised form
10 April 2017
Accepted 14 April 2017
Available online 18 April 2017

Chemical compounds studied in this article:
Desipramine (PubChem CID: 2995)
Fluoxetine Hydrochloride (PubChem CID: 62857)

Keywords:
Desipramine
Fluoxetine
Mitochondria
Functional proteomics
Brain energy metabolism

ABSTRACT

Alterations in mitochondrial functions have been hypothesized to participate in the pathogenesis of depression, because brain bioenergetic abnormalities have been detected in depressed patients by neuroimaging *in vivo* studies. However, this hypothesis is not clearly demonstrated in experimental studies: some suggest that antidepressants are inhibitors of mitochondrial metabolism, while others observe the opposite.

In this study, the effects of 21-day treatment with desipramine (15 mg/kg) and fluoxetine (10 mg/kg) were examined on the energy metabolism of rat hippocampus, evaluating the catalytic activity of regulatory enzymes of mitochondrial energy-yielding metabolic pathways. Because of the micro-heterogeneity of brain mitochondria, we have distinguished between (a) non-synaptic mitochondria (FM) of neuronal perikaryon (post-synaptic compartment) and (b) intra-synaptic light (LM) and heavy (HM) mitochondria (pre-synaptic compartment).

Desipramine and fluoxetine changed the catalytic activity of specific enzymes in the different types of mitochondria: (a) in FM, both drugs enhanced cytochrome oxidase and glutamate dehydrogenase, (b) in LM, the overall bioenergetics was unaffected and (c) in HM only desipramine increased malate dehydrogenase and decreased the activities of Electron Transport Chain Complexes.

These results integrate the pharmacodynamic features of desipramine and fluoxetine at subcellular level, overcoming the previous conflicting data about the effects of antidepressants on brain energy metabolism, mainly referred to whole brain homogenates or to bulk of cerebral mitochondria. With the differentiation in non-synaptic and intra-synaptic mitochondria, this study demonstrates that desipramine and fluoxetine lead to adjustments in the mitochondrial bioenergetics respect to the energy requirements of pre- and post-synaptic compartments.

1. Introduction

The historically leading theory for the pathogenesis of depression is the biogenic amine hypothesis, that was suggested in 1960s because of the decreased concentrations of norepinephrine (NE) and 5-hydroxytriptamine (5-HT) observed in the brains of depressed patients (Crossland, 1963; Kety, 1963). The current main pharmacological therapies are still based on this hypothesis, as reviewed by Ferrari and Villa (2017).

However, depressive disorders are heterogeneous diseases and therapy of depression is not devoid of concerns, including: (i) the time-lag between the acute pharmacological effect, *i.e.* the increase of neurotransmitter brain concentrations, and the therapeutic

Abbreviations: 5-HT, 5-hydroxytryptamine; AD, Antidepressant Drugs; CBF, Cerebral Blood Flow; CCRS, NADH-cytochrome *c* Reductase Rotenone-Sensitive; CCRT, NADH-cytochrome *c* Reductase as Total; CMR_{glu}, Cerebral Metabolic Rate for Glucose; CNS, Central Nervous System; COX, Cytochrome Oxidase; CS, Citrate Synthase; ETC, Electron Transport Chain; FM, Non-synaptic Mitochondria; FST, Forced Swimming Test; GlDH, Glutamate Dehydrogenase; HM, Intra-synaptic Heavy Mitochondria; LM, Intra-synaptic Light Mitochondria; MDH, Malate Dehydrogenase; NE, Norepinephrine; NGF, Nerve Growth Factor; OGI, Oxygen-Glucose Index; PET, Positron Emission Tomography; rCBF, Regional Cerebral Blood Flow; SA, Specific Activity; SD, Sprague-Dawley; SDH, Succinate Dehydrogenase; SSRI, Selective Serotonin Reuptake Inhibitor; TCA, Tricyclic Antidepressant.

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http://dx.doi.org/10.1016/j.neuropharm.2017.04.025
0028-3908/

efficacy, respectively occurring within hours and after weeks of treatment, and (ii) the presence of treatment-resistant depression, accounting for 30–40% of clinical cases. Therefore, new hypotheses and therapeutic strategies are needed.

In this context, many studies observed that mood disorders are associated with alterations in the intracellular signal transduction pathways originating from the activation of NE or 5-HT receptors (Brunello and Tascedda, 2003; Duman et al., 1997; Lenox et al., 1998; Manji et al., 1995, 1996; Perez et al., 2000; Popoli et al., 2000; Racagni et al., 1992). Consequently, the original biogenic amine hypothesis has been updated to include changes in down-regulation and desensitization of pre- and post-synaptic NE and 5-HT receptors (Hamon and Blier, 2013). Moreover, because many molecules of ATP are needed for the activation of the intracellular signaling pathways triggered by the binding of neurotransmitters to their receptors, an increasing interest has been developed about the bioenergetic alterations of the cerebral tissue in mood disorders (reviewed by Moretti et al., 2003). This has lead to formulate the mitochondrial pathogenetic hypothesis of depression (Adzic et al., 2016; Ferrari and Villa, 2017; Wang and Dwivedi, 2016).

At present, proteomic studies performed on after death brains of depressed patients indicate that mood disorders share about 21% of modified proteins, being these proteins mostly related to deregulation of energy metabolism pathways (Saia-Cereda et al., 2016). In addition, neuroimaging *in vivo* studies on human depressed patients reported some modifications of brain energy metabolism, *i.e.* changes in Cerebral Blood Flow (CBF) and Cerebral Metabolic Rate of glucose (CMR_{glu}) (Drevets, 1999, 2000; Price and Drevets, 2012; Stoll et al., 2000). In particular, in the complex pattern of the detected abnormalities, the following neuroimaging findings may be summarized: (i) the thalamus and amygdala are hypermetabolic, while (ii) the anterior cingulate cortex, prefrontal cortex and hippocampus are hypometabolic. As a consequence, the control exerted by the cerebral cortex and hippocampus towards the amygdala does not properly function in depression, and a long-lasting stress-activated *status* is likely established. The resulting persistent glucocorticoid release sustains this vicious cycle and may cause: (i) cortical and hippocampal atrophy and (ii) the monoamine depletion as an adapting response (Ferrari and Villa, 2017).

Therefore, the frontal cerebral cortex and hippocampus seem to be primarily involved in energy metabolism abnormalities in depression (Detka et al., 2015) and the aim of the present research was to evaluate the effects of sub-chronic 21-day pharmacological treatment with desipramine (a tricyclic antidepressant, TCA) and fluoxetine (a selective serotonin reuptake inhibitor, SSRI) on brain energy metabolism of rat hippocampus. The effects of these drugs on rat frontal cerebral cortex have been previously assessed in the same experimental settings (Villa et al., 2016).

The energy metabolism has been studied assaying the catalytic activities of regulatory enzymes of mitochondrial energy-yielding metabolic pathways (functional proteomics). In fact, enzyme activities are indicative of the cerebral tissue ability to respond efficiently (i) to pathological *noxae* and (ii) to pharmacological treatments (Ferrari et al., 2015; Villa et al., 1992, 2013a, 2013b). Moreover, (iii) enzyme activities may be the direct molecular targets of drugs (Moretti et al., 2011, 2015a, 2015b; Villa and Gorini, 1997; Villa et al., 2012a).

In this context, it is of interest the possibility of distinguishing brain mitochondria according to their *in vivo* localization in different sub-cellular neuronal compartments, in the perspective of evaluating the effects of drugs diversifying between pre- and post-synaptic terminals. Therefore, because of this micro-heterogeneity of brain mitochondria (Villa and Gorini, 1991; Villa et al., 1989, 2012b, 2013a), this research was performed on: (i) non-synaptic mitochondria of neuronal perikaryon, *in vivo* located within the post-synaptic compartment, and (ii) intra-synaptic light and heavy mitochondria (two types), *in vivo* located in the pre-synaptic compartment.

This technology was previously proven to be useful when evaluating the effects of the antidepressants desipramine and fluoxetine in the frontal cerebral cortex, where non-synaptic and intra-synaptic mitochondria underwent different modifications (Villa et al., 2016): (a) cytochrome oxidase activity was increased in non-synaptic mitochondria, while (b) malate dehydrogenase, succinate dehydrogenase and glutamate-pyruvate transaminase activities were decreased in intra-synaptic ones. Therefore, it is of interest to evaluate the bioenergetic modifications induced by these antidepressants also in rat hippocampus, in the perspective of confirming the validity of the employed functional proteomic approach.

In fact, this sub-cellular study may overcome the conflicting data so far obtained about the action of antidepressants on mitochondrial energy metabolism, that has been previously evaluated only on pooled mitochondria. For example, Souza et al. (1994) observed that fluoxetine *in vivo* administration stimulated the rat liver mitochondrial state 4 respiration for α-ketoglutarate or succinate oxidations, and this uncoupling effect of oxidative phosphorylation was described also in rat brain mitochondria after the administration of TCAs and other psychotropic drugs (Abdel-Razaq et al., 2011; Byczkowski and Borysewicz, 1979; Ferreira et al., 2014; Fromenty et al., 1989; Weinbach et al., 1986).

On the other hand, imipramine increased the intra-mitochondrial content of cytochrome *b and* $c + c_1$ after 1 week of treatment and that of aa_3 cytochrome after 2 week of treatment (Katyare and Rajan, 1995). Also nortriptyline was identified as a strong inhibitor of mitochondrial permeability transition and likely for this reason is neuroprotective in *in vitro* and *in vivo* models of cerebral ischemia (Zhang et al., 2008). Recently, Filipović et al. (2017) showed that fluoxetine treatment increased the energy metabolism towards the citric acid cycle and oxidative phosphorylation in a mitochondrial proteome study. Therefore, by taking into account the micro-heterogeneity of cerebral mitochondria populations, it would be possible to cast new insights into the molecular mechanisms of action of antidepressant drugs.

2. Materials and methods

2.1. Care of the animals and pharmacological treatment

The experiments were performed on male CD Sprague-Dawley (SD) rats (Charles-River). The animals were kept from birth under standard cycling and housing conditions (temperature: 22 ± 1 °C; relative humidity 60± 3%; lighting cycle: 12 h light and 12 h darkness; low noise disturbances), fed with a standard diet in pellets with water *ad libitum*.

The selection of the animals for pharmacological treatment was established by Fisher and Yates permutation tables, and the rats were divided in three experimental lots: (a) control animals treated with saline physiological solution; (b) animals treated with desipramine (desmethylimipramine; 3-(10,11-dihydro-*5H*-dibenzo[*b*,*f*] azepin-5-yl)-*N*-methylpropan-1-amine) at the dose of 15 mg/kg b.w. per day, by intraperitoneal injection; (c) animals treated with fluoxetine hydrochloride ((*RS*)-*N*-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy] propan-1-amine) at the dose of 10 mg/kg b.w. per day, by intraperitoneal injection. The pharmacological treatment was started from the 7th week of age and continued for 21 days, so to take into consideration the known time-lag between the pharmacological and therapeutic effect of these drugs.

At the end of treatments, at the 10th week of age, the animals were sacrificed under anesthesia by ether (Merck Darmstadt,